HEART LESIONS IN MICE WITH EXPERIMENTAL COXSACKIE A13 VIRUS INFECTION

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The results of pathomorphological and virological investigations in BALB/c mice infected with Coxsackie A13 virus are described. Chronic myocarditis, endocarditis, and valvulitis were discovered in mice infected 10-12 h after birth. The virus was found in the mice only for 15-20 days after infection.

KEY WORDS: Coxsackie virus; myocarditis; endocarditis; valvulitis.

There is much clinical and experimental evidence to show that viruses, including certain members of the Coxsackie group, can induce heart lesions and that most frequently diffuse or focal myocarditis is found in addition to endo- and pericarditis [1-6, 9]. Isolated experimental investigations have shown that Coxsackie B4 virus can cause damage to the valvular apparatus of the heart in mice and primates [7, 8]. However, many representatives of this group of viruses and, in particular, Coxsackie A viruses, have been inadequately studied in this respect.

The object of this investigation was to study the dynamics of pathomorphological changes in the heart in mice experimentally infected with Coxsackie A13 virus.

EXPERIMENTAL METHOD

Newborn (10-12 h after birth) and sexually mature (2-2.5 months) BALB/c mice were used. The animals were infected intramuscularly with the prototype strain (Flores) of Coxsackie A13 virus. The virus was injected in a dose of 15 CPD_{50}/mg live weight of each animal. Mice of

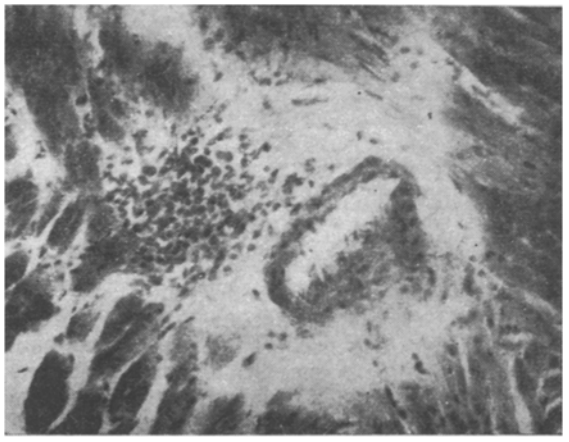

Fig. 1. Perivascular focus of lympho-histiocytic infiltration. Hematoxylin–eosin, 300 ×.

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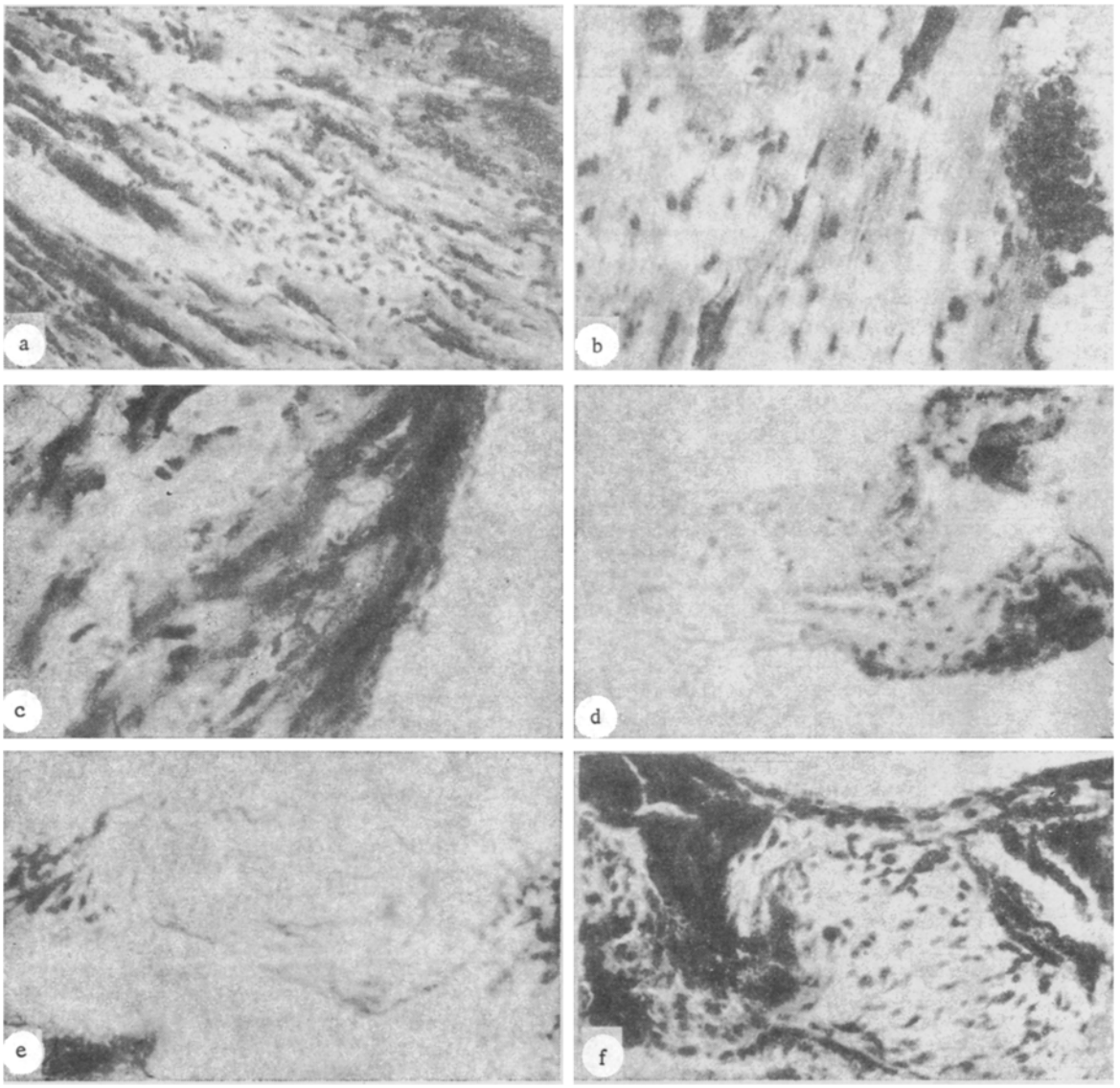

Fig. 2. Heart changes in experimental Coxsackie A13 virus infection: a) interstitial diffuse myocarditis (300 ×); b) subendocardial granuloma (300 ×); c) focal cardiosclerosis (300 ×); d) focus of fibrinoid necrosis in the substance of a valve (280 ×); e) sclerosing granuloma in the substance of a valve (280 ×); f) focal sclerosis of valve (280 ×). a,b,d,e) Stained with hematoxylin–eosin; c,f) stained by Van Gieson's method.

the control group received heat-inactivated virus, culture fluid, and medium No. 199. Intact animals of the corresponding age also were investigated. The mice were killed 1, 3, 5, 7, 11, 15, and 20 days and 1, 3.5, and 5 months after infection. At each of these times 8-12 animals were investigated virologically and histologically. Virus from the heart tissue and from other materials from the infected animals was isolated and titrated in a culture of human embryonic fibroblasts. The material for histological and histochemical investigation was fixed in neutral formalin, stained with hematoxylin–eosin by Van Gieson's method, sections were stained selectively with toluidine blue at different pH values, the PAS reaction was carried out with appropriate enzyme controls, and sections were impregnated by Gomori's method.

EXPERIMENTAL RESULTS

In mice infected with the virus in the neonatal period the first clinical signs of the disease characteristic of the infection produced by Coxsackie viruses usually appeared on the first to third days after infection. No signs of disease were found in any of the mice infected at the age of 2 months at any time of the investigation.

Virus was found in the blood of the infected newborn animals by the subculture method until 9 days after infection, and in the heart until 7 days. When adult animals were infected, the times during which virus could be found in the blood and heart were greatly reduced (to 1 and 2 days). The virus was detectable for longest in the skeletal muscles of the animals (until 15 days after infection in sexually mature mice and until 20 days in newborn mice).

Microscopic examination of heart sections at various times (1, 3, 5, and 7 days) showed evidence of disturbance of the circulation and predominantly alterative changes.

On the third, fifth, and seventh days of development of the infection the circulatory disturbances and degenerative changes in the heart of the infected animals reached their maximum, and on the 11th and 15th days they had subsided a little. The sarcoplasm of the cardiac myocytes of the animals 20 days after infection was unevenly stained. Areas stained a deep pink color were found, together with paler, granular areas. The nuclei of the muscle cells as a rule were large. Small collections of lymphocytes and histiocytes were observed initially in some areas of the stroma, chiefly in the perivascular and subendocardial regions. Somewhat later (on the 31st-34th day after infection) large foci of lymphohistiocytic infiltration of the granuloma type were observed, mainly in the perivascular and subendocardial regions of the heart (Figs. 1 and 2b). Sometimes granulomas of this sort were found in the substance or at the base of the heart valves. Signs of degenerative changes still persisted in the myocytes. The endocardium was thickened and homogeneous, and foci of proliferation of the endothelial cells lining the endocardium resulted in the formation of localized thickenings.

In the final periods of observation (3.5 and 5 months) these patterns of inflammatory changes in the heart of the infected animals continued, but they were joined by scar changes. Against the background of focal myocarditis, sometimes pictures of widespread lymphohistiocytic infiltration of the interstitial tissue of the myocardium (Fig. 2a) could be observed at these times of the investigation (just as after 1 month), and they were regarded as a manifestation of diffuse myocarditis.

After staining with toluidine blue, a weak degree of γ metachromasia was observed at places of thickening of the endocardium, in the perivascular region, and in the region of the granulomas. In sections stained by Van Gieson's method excessive proliferation of collagen fibers was seen, mainly around the myocardial vessels. Single collagen fibers were seen between the myocytes and also in connective-tissue layers between the bundles. Collections of very delicate collagen fibers could be seen in some parts of the granulomas. Excessive proliferation of collagen fibers also was observed in the endocardium (Fig. 2c).

In the heart valves of the animals of both experimental groups in the late stages of the investigation (after 1-5 months) substantial changes also were found. The valves were irregularly thickened and discrete foci of fibrinoid necrosis could be seen in their substance (Fig. 2d). Clusters of lymphohistiocytes and marked proliferation of fibroblasts were sometimes observed around these foci. Proliferation of the valvular endothelium was present, but no disturbances of the endothelial lining could be seen. In sections stained by Van Gieson's method foci of proliferation of collagen fibers, corresponding to the positions of old granulomas, could be seen in the valves (Fig. 2f). In these situations of granulomas, γ metachromasia was observed after staining with toluidine blue. The PAS reaction was positive in the areas of necrosis, especially at the base of the valves.

In foci of cicatrization of the granulomas, and in the perivascular and subendothelial regions, excessive proliferation of collagen fibers was observed.

In some areas of the myocardium, collagen fibers were revealed by Van Gieson's method between the muscle fibers; in sections stained by Gomori's method some coarsening of the argyrophilic skeleton of the myocardium was observed.

No histological evidence of a heart lesion could be found in any of the mice of the control groups studied.

Valvulitis, accompanied by granulomatous myocarditis and endocarditis, with the features of chronic inflammation, was thus observed in the heart of BALB/c mice with experimental Coxsackie A13 virus infection. In all probability these phenomena may lie at the basis of the substantial disturbances of cardiac function. The results confirm existing data [4, 6] showing that Coxsackie virus can produce chronic lesions in the heart.

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